



Atty. Dkt. No. DOCKET NO. 50939/104

In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 3, lines 12-13, and replace it with the following paragraph:

FIG. 2(a) provides the *papH* DNA sequence of pHUR849 (SEQ ID NO: 31), FIG. 2(b) pDAL201B *papH* (SEQ ID NO: 33), FIG. 2(c) pDAL210B *papH* (SEQ ID NO: 35), and FIG. 2(d) pDAL200A (SEQ ID NO: 37).

Please delete the paragraph on page 3, lines 14-15, and replace it with the following paragraph:

FIG. 3 provides a comparison of the *papH* DNA sequences of pHUR849 (SEQ ID NO: 31), pDAL200A (SEQ ID NO: 37), pDAL201B (SEQ ID NO: 33), and pDAL210B (SEQ ID NO: 35).

Please delete the paragraph on page 3, lines 16-17, and replace it with the following paragraph:

FIG. 4 gives a comparison (SEQ ID NO: 39) of deduced amino acid sequences of *papH* genes for pHUR849, pDAL200A, pDAL201B, and pDAL210B.

Please delete the paragraph on page 3, lines 18-20, and replace it with the following paragraph:

FIG. 5(a) shows the amino acids (which are underlined) that are deleted from *papH* in pHUR849 (SEQ ID NO: 32) and FIG. 5(b) shows the amino acids (which are

underlined) that are deleted from papH in pDAL201B (SEQ ID NO: 34), pDAL210B (SEQ ID NO: 36), and pDAL200A (SEQ ID NO: 38).

Please delete the paragraph on page 7, lines 18-24, and replace it with the following paragraph:

The following peptide conjugate vaccines were demonstrated to be protective after serial parenteral administration in the BALB/c murine model of experimental pyelonephritis after intravesicular administration:

C 5

<u>F serotype</u>	<u>Structural Pilin Sequence</u>	<u>Residue Position (R)</u>
F13 (<u>SEQ ID NO: 1</u>)	PQGQQGKVT	R 5-12
F13 (<u>SEQ ID NO: 2</u>)	AKFGGMGAKKG	R 65-65

Please delete the paragraph on page 8, lines 21-34, and replace it with the following paragraph:

The following peptide thyroglobulin and bovine serum albumin conjugate vaccines were made:

C 6

<u>F serotype</u>	<u>Structural Pilin Sequence</u>	<u>Residue Position (R)</u>
F71 (<u>SEQ ID NO: 3</u>)	PQGQQGEVSF	R 5-12
F71 (<u>SEQ ID NO: 4</u>)	NFKQLQGGAAKKG	R 65-77
F72 (<u>SEQ ID NO: 5</u>)	PQGQQGKVT	R 5-12
F72 (<u>SEQ ID NO: 6</u>)	NFKKAAGGGGAKT	R 65-75
F9 (<u>SEQ ID NO: 7</u>)	QGSGQVNFKG	R 4-12
F9 (<u>SEQ ID NO: 8</u>)	NFKKAATPGGAAKT	R 65-75
F11 (<u>SEQ ID NO: 9</u>)	IPQQGQGKVTFNG	R 4-15
F12 (<u>SEQ ID NO: 10</u>)	IPEGQGKVT	R 2-12
F1C (<u>SEQ ID NO: 11</u>)	NGGTVHFKGEVVN	R5-12
F1 (<u>SEQ ID NO: 12</u>)	TTVTVNNGGTVHF	R4-15

Please delete the paragraph on page 10, lines 5-18, and replace it with the following paragraph:

Results indicated the following:

<u>F serotype</u>	<u>Pilin A Sequence</u>	<u>Residue Positions</u>	<u>Homologous Protection</u>
{ F71 (<u>SEQ ID NO: 13</u>)	PQQQGEVT	R 5-12	Yes
{ F71 (<u>SEQ ID NO: 14</u>)	PQQQGEVA	R 5-12	Yes
{ F71 (<u>SEQ ID NO: 4</u>):	NFKQLQGGAAKKKG	R 65-77	Yes
{ F72 (<u>SEQ ID NO: 5</u>)	PQQQGKVT	R 5-12	Yes
{ F72 (<u>SEQ ID NO: 6</u>)	NFKKAAGGGGAKT	R 65-77	Yes
{ F9 (<u>SEQ ID NO: 15</u>)	TTVNGGTvh	R 4-12	Yes
{ F9 (<u>SEQ ID NO: 8</u>)	NFKKAATPGGAAKT	R 65-75	Yes
F11 (<u>SEQ ID NO: 16</u>)	IPQQQGKVTFNGTV	R 4-17	Yes
F12 (<u>SEQ ID NO: 10</u>)	IPEGQQGKVT	R 4-12	Yes
F1C (<u>SEQ ID NO: 11</u>)	NGGTvHFKGEVVN	R 5-15	Yes
F1 (<u>SEQ ID NO: 12</u>)	TTVTvNGGTvHF	R4-15	Yes

Please delete the paragraph on page 10, lines 20-41, and replace it with the following paragraph:

One or a combination of pilin A vaccines comprising one or more of the following amino acid sequences that correspond to published and unpublished F pilin primary sequences would be protective against ascending, non-obstructive *Escherichia coli* urinary tract infections in anatomically normal women and males:

<u>F serotype</u>	<u>Pilin A Sequence</u>	<u>Positions</u>	<u>Pilin A Residue Urinary Tract</u>	<u>New or Old Claim</u>
4	F71 (SEQ ID NO: 13) PQGQGEVT	R 5-12	Pyelonephritis	New
C8 2	P71 (SEQ ID NO: 14) PQGQGEVA	R 5-12	Pyelonephritis	New
3	F71 (SEQ ID NO: 4) NFKQLQGGAAKKKG R65-77		Pyelonephritis	New
1	- F72 (SEQ ID NO: 5) PQGQGKVT	R 5-12	Pyelonephritis	New
2	F72 (SEQ ID NO: 6) NFKKAAGGGGAKT R65-77		Pyelonephritis	New
1	- F9 (SEQ ID NO: 15) TTVNGGTvh	R 4-12	Pyelonephritis	New
2	F9 (SEQ ID NO: 8) NFKKAATPGGAAKT R 65-75		Pyelonephritis	New
1	- F11 (SEQ ID NO: 16) IPQQQGKVTFNGTV	R 4-17	Pyelonephritis	New
2	- F12 (SEQ ID NO: 10) IPEGQGKVT	R 4-12	Pyelonephritis	New
1	- F13 (SEQ ID NO: 1) PQGQGKVT	R 5-12	Pyelonephritis	Old
2	F13 (SEQ ID NO: 17) AKFGGMGAKKG	R 65-65	Pyelonephritis	Old
1	- F1C (SEQ ID NO: 11) NGGTVHFKGEVVN	R 5-15	Cystitis	New
1	- F1 (SEQ ID NO: 12) TTVTVNGGTvHF	R4-15	Cystitis	New

Please delete Table 2 on page 19 and replace it with the following Table:

TABLE 2. Primers used in this study

Primers	Oligonucleotide sequence	Description
T3	5' ATTAACCCTCACTAAAG 3' <u>(SEQ ID NO: 18)</u>	anneals to multiple cloning site of SK-
T7	5' AATACGACTCACTATAG 3' <u>(SEQ ID NO: 19)</u>	anneals to multiple cloning site of SK-
Reverse	5' AACAGCTATGACCATG 3' <u>(SEQ ID NO: 20)</u>	anneals to multiple cloning site of SK-
PGpHFD	5' ATGAGACTGCGATTCTCTGT 3' <u>(SEQ ID NO: 21)</u>	anneals to the TAC translational start region of all 4 <i>pap H</i> genes
PapHRE	5' TCCGTTCTCACAAATTCTGA 3' <u>(SEQ ID NO: 22)</u>	anneals to bp 509-528 of the <i>pap H</i> gene of pDAL201B, <i>pap-21</i> and pHUR 849, <i>pap-5</i>
210bFD	5' CCTGAAATACGAGAAATTAA 3' <u>(SEQ ID NO: 23)</u>	anneals 93-bp upstream of the TAC translational start region of the <i>pap A</i> gene of pHUR849, <i>pap-5</i> (2)
210bRE	5' TAATATCTCGTATTCAGG 3' <u>(SEQ ID NO: 24)</u>	the complement of 210bFD and anneals to the same 93-bp region as described for 210bFD
FOR210b	5' TGGACTGGTATAACAATCGA 3' <u>(SEQ ID NO: 25)</u>	anneals 2.9 kb upstream of the TAC translational start region of the <i>pap H</i> gene of pDAL210B, <i>pap-21</i>
200aRE	5' TCCGTTTCGCACAATTCTGA 3' <u>(SEQ ID NO: 26)</u>	anneals to bp 511-528 of the <i>pap H</i> gene of pDAL2I OB, <i>pap-17</i> , and <i>pap 200a</i> , respectively
PapFOR ^a	5' AG <u>GGGATT</u> CATGCAGCATTCT AGAAA 3' <u>(SEQ ID NO: 27)</u>	anneals to bp 258-270 of the <i>pap A</i> gene of pHUR849, <i>pap-5</i> (2)
FORSEQ	5' TGGACCTCCTGAGCTA 3' <u>(SEQ ID NO: 28)</u>	anneals to bp 456-474 of the <i>pap A</i> gene of pHUR849, <i>pap-5</i> (2)
PapREV ^b	5' GGGCAGCCCTGCCGTCCCAA AT 3' <u>(SEQ ID NO: 29)</u>	anneals to bp 122-142 of the <i>pap H</i> gene of pHUR849, <i>pap-5</i>
REVSEQ	5' AAACACCATGAAACACACA 3' <u>(SEQ ID NO: 30)</u>	anneals to bp 41-61 of the <i>pap H</i> gene of pHUR849

^a contains a single Bam HI restriction site single underlined.

^b contains a single Sma I blunt end restriction site double underlined.

Please delete the paragraph on page 22, line 5 to page 23, line 6 and replace it with the following paragraph:

Nucleotide Sequences and Deduced *PapH* Primary Structures

The plasmids pHUR849 (*pap*-5), pDAL201B (*pap*-21), pDAL210B (*pap*-17) and, pDAL200A (*pap*-200A), in *E. coli* strain HB101 express digalactose-binding of the serotypes F13, F7₁, F7₂ and F9, respectively. The *pap* gene cluster responsible for regulation and biogenesis of these pili from *E. coli* strains J96, C1212 and, 3669 is 1U. diagrammed in FIG. 1. Sequence analysis of *papH* genes from pDAL201B (*pap*-21), pDAL210B (*pap*-17) and, pDAL200A (*pap*-200A), was compared to the known nucleotide sequence of *papH* gene of pHUR849 (*pap*-5) (3). FIG. 2 shows a single 588-bp open reading frame with the same polarity as *papA* (2, 4). Analyses of these *papH* sequences revealed many typical features of prokaryotic gene organization. All four *papH* gene sequences contained a potential ribosome-binding sites, ATG initiation codon signal sequence, and a TGA termination codon. A potential initiation codon ATG at position -22, preceded by a sequence corresponding to -AGGGT, which showed homology to ribosome-binding sites, was found 13-bp upstream in all four *papH* sequences. A protein initiated here and ending at the TGA triplet at position 586 would encode a 195 amino acid polypeptide with a calculated molecular weight of 21.9 kd. The mature *PapH* protein contains 173 amino acid residues. The NH₂-terminal amino acid sequence of the open reading frame has all the features of a signal peptide sequence. The deduced putative signal sequence for the *papH* was located 22 codons upstream of their terminal Ala (FIG. 2). These sequences contained a highly hydrophobic region comprising an amino acids stretch of Ser-Val-Pro-Leu-Phe-Phe (residues -17 to -11 of SEQ ID NO: 32). There was a positively charge amino acid residue (Arg) at the position -21. The suggested cleavage sites between Ala -1 and gly +1 conforms to rules of prokaryotic signal cleavage sites and was similar to most other bacterial genes (12). In addition, the final *papH* deletion derivatives, pKD849-5 (*pap*-5), pKD201B (*pap*-21), pKD210B-1 (*pap*-17) and pKD200A-8 (*pap*-200A), were also sequenced. In addition, sequencing into the *papA* and *papC* genes which flank the *papH* gene (FIG. 1) of all four *papH* deletion derivatives was carried out in order to insure that all three genes were in frame. Finally, the codon usage of the *papH* genes of pDAL201B, pDAL210B and, pDAL200A, and *papH* gene of pHUR849 were analyzed using a codon frequency computer program (13). The pattern of codon utilization was not significantly different among the genes.